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Effects of a "step-up" ractopamine feeding program, sex, and social rank on growth performance, hoof lesions, and Enterobacteriaceae shedding in finishing pigs[1]

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ABSTRACT: Increasing concern for animal well-being and food safety has stimulated the investigation of feed additives such as ractopamine (RAC), a β-agonist widely used to improve production performance of finishing pigs. The objective of this study was to determine effects of RAC feeding, delivered as a "step-up" program (5 mg/kg for 2 wk followed by 10 mg/kg for 2 wk), on growth performance, Enterobacteriaceae shedding, including *Salmonella*, and hoof lesions, also taking into account sex and social rank of pigs. A total of 64 barrows and gilts (balanced by treatment and sex) were assigned to pens of 4 (by sex) as either control (CTL) or RAC treatment. Social ranks (dominant, intermediate, and subordinate) of pigs in each pen were determined by behavioral observation during 48 h postmixing. Fecal samples were collected once per week for 5 wk. At slaughter, the 32 dominant and subordinate barrows and gilts (16/sex) were examined for hoof lesions, and luminal contents from ileum, cecum, and rectum were collected. Pigs fed RAC had increased growth performance ($P < 0.05$) with social rank of animals affecting overall ADG ($P < 0.05$). Gilts gained more backfat than barrows when comparing to baseline values at both 10th and last ribs ($P < 0.05$), whereas loin eye area increased at a similar rate for both barrows and gilts ($P > 0.10$). No significant effect of RAC feeding was found on backfat or loin eye area ($P > 0.10$). At slaughter, RAC-fed pigs had greater BW ($P < 0.05$). Despite the positive effects of RAC feeding on growth performance, pigs fed the compound had a greater frequency of front and rear hoof lesions as did barrows and dominant individuals ($P < 0.05$). Detectable concentrations of *Salmonella* shedding were not identified at any time during the experiment. Enterobacteriaceae shedding concentrations from RAC-fed pigs peaked at the first week of feeding and progressively decreased until slaughter. At slaughter, rectal and cecal Enterobacteriaceae concentrations were less in RAC-fed pigs than in CTL pigs ($P < 0.05$). Social rank tended to affect gut Enterobacteriaceae populations of barrows more than in gilts ($P < 0.10$). The effects of RAC feeding on hoof soundness and Enterobacteriaceae populations in the gastrointestinal tract of finishing pigs warrant further investigation. It is also proposed that the integration of the social rank status of the individual into future studies should be considered, because it may affect treatment responses.

Key words: Enterobacteriaceae, hoof soundness, performance, ractopamine, social rank, swine

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INTRODUCTION

The β-adrenergic agonist ractopamine (**RAC**) is increasingly used in the US swine industry for its enhancing effects on growth performance in finishing pigs. As a repartitioning agent, RAC promotes lean tissue deposition (Mills and Spurlock, 2003) and improves feed efficiency and growth (Gu et al., 1991a,b). The legal label for RAC feeding ranges between 5 to 10 mg/kg, fed for the last 18 to 40 kg before slaughter at a constant concentration or as a "step-up" feeding program. Increasing RAC feeding concentrations after 2 wk of sustained dose optimizes its effects that otherwise begin decreasing (Williams et al., 1994). Opposing the beneficial effects of RAC on swine production are some negative behavioral consequences of its feeding, such as hyperactivity, overreactivity to transport, and difficulty of handling (Marchant-Forde et al., 2003). Addition-

[1]Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the USDA and does not imply approval to the exclusion of other products that may be suitable.

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ally, RAC is linked to physiological changes such as elevated heart rate and increased catecholamine concentrations that are indicative of stress (Marchant-Forde et al., 2003).

Other issues that have not been largely addressed in swine fed RAC include hoof condition and gut microbiology. As animals receive RAC, the rapid increase in BW and physical activity may lead to greater frequency of hoof lesions with potential effect on well-being and productivity. Salbutamol, another β-adrenergic agonist, leads to gait problems due to its effect on hoof soundness (Penny et al., 1994). Increasing concern for food safety also warrants investigation into the effects of RAC on potential foodborne pathogens included in the Enterobacteriaceae family, particularly *Salmonella.* Scarce information is found on the potential effects of RAC on the microbial populations of the gastrointestinal tract (Edrington et al., 2006a,b). Therefore, our objectives were to evaluate growth performance, microbial responses, and hoof integrity of pigs subjected to a "step-up" RAC feeding program and to further determine effects of sex and social rank on the responses above.

Table 1. Composition of the experimental basal diet provided to late finishing pigs, as-fed basis

Item	Control
Ingredient, %	
Ground corn	67.23
Soybean meal, 48% CP	25.00
Choice white grease	5.00
Ground limestone	0.85
Dicalcium phosphate	0.75
Vitamin[1] and trace mineral premix[2]	0.30
Phytase[3]	0.10
Salt	0.25
L-Lysine·HCl	0.22
DL-Methionine	0.10
L-Threonine	0.10
Microaid[4]	0.05
Starch[5]	0.05
Ractopamine·HCl[5]	0.00
Calculated analysis	
ME, kcal/kg	3,543.00
CP, %	17.63
Lysine, %	1.10
Methionine, %	0.38
Methionine + cysteine, %	0.69
Threonine, %	0.76
Tryptophan, %	0.20
Ca, %	0.60
P, %	0.50
Estimated available P, %	0.29

[1]Provided per kilogram of diet: vitamin A, 3,638 IU; vitamin D_3, 364 IU; vitamin E, 26.4 IU; menadione, 1.20 mg; vitamin B_{12}, 21 µg; riboflavin, 4.26 mg; pantothenic acid, 13.20 mg; and niacin, 19.80 mg.

[2]Provided per kilogram of diet: Fe, 97.0 mg; Zn, 97.0 mg; Mn, 12.0 mg; Cu, 9.0 mg; I, 0.37 mg; and Se, 0.30 mg.

[3]Provided per kilogram of diet: 600 phytase units.

[4]Yucca extract product.

[5]Ractopamine HCl (Paylean-9, Elanco Animal Health, Greenfield, IN) was added at the expense of starch to create diets with 5 and 10 mg/kg of ractopamine HCl.

MATERIALS AND METHODS

The experiment was approved by the Purdue University Animal Care and Use Committee, and animals were housed in accordance with FASS (1999) guidelines.

Animals and Housing

A total of 64 finishing pigs including 32 barrows and 32 gilts [(US Duroc × Hampshire) × (US York × Landrace)] were used. Experimental pigs were selected from a population of 170 animals according to initial BW and ancestry and were assigned to 1 of 4 BW blocks. Blocks 1 and 2 began the study 2 wk apart from blocks 3 and 4. Each block was balanced for sex, treatment, and BW (without restriction of feed and water) and was allocated into 1 of 2 rows of 8 adjacent pens, which housed 4 pigs per pen. Both rows of pens were located in the same room of the Purdue University Swine Evaluation Unit and were separated by a 1.5-m-wide aisle. Pens in each row were organized so they alternated sex and treatment order within a BW block. This was done to remove any potential location effects within the barn. Each pen was 1.8 m × 3.0 m and had the rear two-thirds of its area as fully slatted concrete floor and the front one-third covered with plastic-coated expanded metal. A single nipple drinker was mounted at the middle of the pen, and a single-spaced feeder was situated at the front of each pen. Animals had water and feed provided ad libitum. The room was ventilated naturally, and temperature was maintained at a minimum of 18.5°C.

Within each block, pens were assigned to either control (**CTL**) or RAC treatment, and at mixing, pigs within each pen were individually identified (beyond individual ear notches) with symbols marked on their backs for behavioral observation. Upon assignment to the experimental pens, pigs on both treatments were fed the same standard basal diet (see Table 1) for 2 wk. Thereafter, CTL pigs continued to receive the standard basal diet, whereas RAC pigs had part of the starch fraction of the basal diet substituted by RAC HCl (see Table 1). Ractopamine was delivered in a "step-up" feeding program, and thus, RAC-fed pigs received the compound added to their diet at 5 mg/kg for 2 wk (phase I: d 0 to 14), and then it was raised to 10 mg/kg for the final 2 wk preceding slaughter (phase II: d 14 to 28). The basal diet was corn-soybean meal-based and feed in meal form (Table 1).

Social Rank

Behaviors of pigs in each pen were continuously recorded from the time of mixing up to 48 h later (d −14 to d −12) using ceiling-mounted cameras (Panasonic WV-CD110AE, Matsushita Electric Industrial Co. Ltd., Osaka, Japan) attached to a digital video recording system (IPD-DVR816, Inter-Pacific Inc., Northbrook, IL). This behavioral information was used to determine dominance hierarchy formation and thus social rank status of animals within their new home pens. In each pen of 4, the top dominant pig and the bottom subordinate pig were determined based on the proportion of encounters that each individual won or lost using a combination of specific measures of social status assignment (Martin and Bateson, 1988; Bradshaw et al., 2000). The remaining 2 pigs in the pen were categorized as intermediate in the social rank. For monitoring the stability of social rank status, behavior was continuously observed for 1 d per week during the period of the greatest activity levels of the animals (0800 to 1100 h), and all instances of social interaction (e.g., aggressive encounters) in each pen were recorded and evaluated.

Animal Growth Performance

Body weights were collected to monitor growth performance. All animals were weighed individually on the arrival day (d −14), on the first day of RAC administration (d 0), on the first day of the third week (d 14), and on the last day of the experiment (d 28, three days before slaughter). Additionally, pen feed allocation was recorded daily and feed remaining in the feeders was weighed back on the same day that the BW of the pigs were measured. Production indicators such as pen average BW, ADG, ADFI, and G:F of CTL and RAC-fed animals were determined from the 2 wk before the beginning (d −14) up to the end of the experiment (d 28). Average BW and ADG were also separately determined for dominant, intermediate, and subordinate pigs. Thus, individual and not pen was considered the experimental unit for these measures when taking into account social rank.

Live Ultrasound Scanning

Live animal B-mode ultrasound (Aloka Model 500V Real-Time Ultrasound, Corometrics Medical Systems, Wallingford, CT) was used to measure backfat depth (**UBFTR**, mm) 7.61 cm off midline and loin eye area (**ULEA**, cm^2) at the 10th rib. Backfat at the last rib (**UBFLR**, mm) was also measured. These measurements were collected twice from all individuals in each pen, once before the beginning of the experiment (d −3, initial scanning) and another 4 wk later, at the last day of the experiment (d 28, final scanning). Both UBFTR and UBFLR in addition to ULEA were evaluated and analyzed as pen averages and also as individual averages when social rank status of animals was taken into account.

Slaughter

After the end of the study (d 31), all animals classified as the dominant and subordinate (n = 32) in each pen were loaded onto a flatbed truck and transported to the slaughter facility at the Purdue University, West Lafayette campus, a distance of approximately 16.1 km, for further sample collection. At the slaughter facility, pigs were off-loaded and held in lairage for up to 1 h preceding slaughter. The 2 remaining intermediate-ranked pigs from each pen were slaughtered at a commercial facility. From the last day of the study (d 28) to the actual slaughter day (d 31), there was a 3-d interval. During this period, RAC pigs continued to be fed ad libitum with the 10 mg/kg of RAC-supplemented diet, whereas CTL pigs were maintained on the same basal diet as given during the 4-wk study. Therefore, BW at slaughter was collected, enabling us to determine BW gain during those 3 d and compare back to BW at d 28 of the study. For the purpose of a neurophysiological study carried out with this same group of animals, pigs had to be skinned as heads were removed immediately postmortem. Therefore, HCW and carcass yield were measured but not reported.

Hoof Lesion Scoring

All individual animals slaughtered at the Purdue University facility also had their front and rear hooves assessed for lesions. Lesions were scored as 3 distinct categories: splits (apparent as separation of the hoof wall away from the underlying layer), cracks-erosions (false sand cracks in the hoof wall and erosions in the solar layer), and bruises (distinct areas of dark discoloration in the sole and behind the hoof wall). Individual lesions in each category were counted, and thus, lesion scores represent a quantitative rather than a qualitative measure of hoof soundness.

Bacteriological Measurements

Individual fecal samples were collected weekly from all animals (n = 64) in each pen (total of 5 samples per pig) to monitor potential effects of RAC on the longitudinal shedding of *Salmonella* and Enterobacteriaceae. Fecal sampling was first performed before the beginning of the experiment (d −3) and continued once per week for the following 4 wk (d 4, 11, 18, and 25) before slaughter. Samples were collected directly from the rectum and immediately stored in sterile plastic bags. Additionally, at slaughter, individual ileal, cecal, and rectal luminal content samples were collected from 32 animals (only dominant and subordinate pigs of each pen). Samples were transported to the laboratory and processed within 2 to 3 h postslaughtering. All samples were individually processed for isolation of *Salmonella* and enumeration of total Enterobacteriaceae. For the *Salmonella* isolation procedure (Rostagno et al., 2005), each sample (1 g) was sequentially enriched in tetrathionate broth (1:10; 37°C for 24 h) and Rappaport-Vassiliadis broth containing 20 μL/mL of novobiocin (1:100; 42°C for 24 h). Subsequently, samples were streaked on xylose lysine tergitol-4 agar and incubated at 37°C for an additional 24 h. For the enumeration of total Enterobacteriaceae, each sample (1 g) was 10-fold diluted and spread on MacConkey agar (100 μL/plate) in duplicate. All plates were incubated at 37°C for 24 h, and colonies were counted. Colony counts (cfu/g) were subjected to logarithm transformation ($\log_{10}$) before statistical analyses.

Statistical Analysis

Pen was used as the experimental unit (n = 16) for statistical analysis of live ultrasound scanning and growth performance measurements such as BW, ADG, ADFI, and G:F. The experiment was set as 4 identical blocks determined by initial BW. Each block consisted of treatment (RAC or CTL) and sex (barrow or gilt) and was balanced in an alternating fashion throughout the barn. Thus, a 2 × 2 factorial analysis with treatments, sex, and their interaction was computed, and repeated measures of pen data were applied.

Social rank (dominant, intermediate, or subordinate) was included as an additional model factor for further analysis of live ultrasound scanning, BW, and ADG using individual pigs as experimental units (n = 64). Data of intermediate pigs in each pen (2 animals per pen) were averaged for this statistical analysis. Additionally, all pigs sent to the Purdue University Slaughter Facility (n = 32; dominant and subordinate animals from each pen) were accounted individually for the statistical analyses of BW at slaughter, hoof lesion scoring averages, in addition to rectal, cecal, and ileal Enterobacteriaceae concentrations as a 2 × 2 × 2 factorial design. Social rank is an individual classification and thus it cannot be taken into account when pen averages are generated. The inclusion of this factor generated a 2 × 2 × 3 factorial design of treatment, sex, and social rank with 4 repeated blocks for the model analyzing fecal Enterobacteriaceae shedding concentrations, considering day of sampling as the repeated measure and individuals as the subjects (n = 64). All the variances were estimated, and model assumptions (e.g., normality) were assessed. Main effects and all possible interactions were computed depending on the significance of the higher-order interactions. Linear mixed models with pairwise comparisons were applied, and P-values of means were adjusted according to the post-hoc test of Tukey using the SAS software (SAS Inst. Inc., Cary, NC). All means, respective SEM, and P-values are presented in tables and figures or described in the text. Mean differences with P-values <0.10 were considered tendencies toward significance, whereas mean differences with P-values <0.05 were considered statistically different.

RESULTS

Growth Performance

Body weight and feed intake are presented as treatment main effects of CTL and RAC and barrows vs. gilts (Table 2), because there was no evidence of significant treatment × sex interactions ($P > 0.10$). Barrows were heavier ($P < 0.05$) than gilts before the initiation of the experimental diets (d −14) and at the beginning day of the trial (d 0; Table 2). Initial pretrial average BW across finishing pigs assigned to RAC and CTL treatments was 77.7 kg ($P > 0.10$). No other growth measurements were considered before initiating the experiment.

Results from the first 2 wk of the experiment, when RAC was fed at 5 mg/kg (phase I: d 0 to 14) did not indicate any differences ($P > 0.10$) in growth performance between CTL and RAC animals; only numerical increases for ADG and G:F for RAC-fed pigs. However, during this phase, barrows were heavier at d 14 ($P < 0.01$) and consumed more feed than gilts (ADFI, $P < 0.01$). During the "step-up" feeding program period in which RAC dose was increased to 10 mg/kg (phase II: d 14 to 28), RAC-fed animals had greater ADG ($P < 0.05$), G:F, and BW at d 28 ($P < 0.01$) compared with CTL pigs. Barrows were heavier than gilts at d 28 ($P < 0.01$), and during phase II, barrows continued to have greater ADFI than gilts ($P < 0.05$). Overall ADG and G:F were greater ($P < 0.01$) for RAC-fed pigs compared with controls, but ADFI did not differ between treatments. Additionally, as an overall sex effect, barrows had greater ADFI ($P < 0.01$) and less G:F ($P < 0.05$) compared with gilts.

Social Rank and Individual Growth Performance. Body weights and ADG were also determined for the pigs in each pen considering the social rank of the individual (Table 3). The other growth performance variables were not determined, because feed intake was measured and averaged by pen and not by consumption by the individual. Results indicate that barrows were consistently heavier than gilts throughout the experiment ($P < 0.05$), but there is no pretrial (d−14) social rank effect on BW ($P > 0.10$). However, the treatment × social rank interaction tended toward significance for BW at d 0 ($P = 0.08$). In the CTL treatment, subordinate pigs were the lightest (90.5 ± 1.2 kg) and the intermediate social rank pigs were the heaviest (94.3 ± 1.2 kg). Meanwhile, in the RAC treatment, the subordinate pigs were the heaviest (94.5 ± 1.2 kg) and the intermediate social rank pigs were the lightest (92.7 ± 1.2 kg). The dominant RAC-fed pigs weighed an average of 93.9 ± 1.2 kg. At phase I, no evidence was found of main effect or interactions involving social rank; however, sex interacted with social rank for phase II ADG ($P = 0.05$). Barrows of intermediate social rank status had greater ADG than that of dominant barrows during phase II ($P < 0.05$). Additionally, for overall ADG, the interaction of sex with social rank tended to follow a similar pattern for ADG as observed during phase II ($P = 0.08$). This interaction was a result of the dominant barrows growing slower than the intermediate barrows (1.08 vs. 0.82 ± 0.06; $P < 0.05$), but ADG for both groups were not different from that of subordinate barrows (1.03 ± 0.06; $P > 0.10$). Meanwhile, gilts had

Table 2. Main effect means for growth performance variables, measured pretreatment, at 14 and 28 d of receiving either control or ractopamine-supplemented diets, and the effects of sex, in finishing pigs

Item	Sex[1] CTL	RAC	Sex[1] Barrow	Gilt	Pooled SEM	Treatment × sex, P-value
Pretreatment						
BW at d −14, kg	77.71	77.73	78.29[c]	77.14[d]	0.19	0.979
BW at d 0, kg	92.70	93.34	94.82[c]	91.22[d]	0.70	0.186
Phase I,[2] d 0 to 14						
ADG, kg/d	0.882	0.993	0.979	0.896	0.047	0.130
ADFI, kg/d	2.992	2.831	3.219[c]	2.604[d]	0.125	0.648
G:F	0.302	0.350	0.309	0.343	0.017	0.130
BW at d 14, kg	105.05	107.24	108.52[c]	103.77[d]	1.00	0.789
Phase II,[2] d 14 to 28						
ADG, kg/d	0.914[a]	1.096[b]	1.004	1.006	0.038	0.265
ADFI, kg/d	2.953	2.987	3.097[c]	2.844[d]	0.075	0.103
G:F	0.311[a]	0.367[b]	0.326	0.351	0.011	0.836
BW at d 28, kg	117.84[a]	122.58[b]	122.58[c]	117.85[d]	0.88	0.646
Overall, d 0 to 28						
ADG, kg/d	0.898[a]	1.044[b]	0.991	0.951	0.021	0.285
ADFI, kg/d	2.973	2.910	3.158[c]	2.724[d]	0.079	0.215
G:F	0.307[a]	0.358[b]	0.318[c]	0.347[d]	0.008	0.134

[a,b]Within treatment rows, means with different superscript letters differ ($P < 0.05$).
[c,d]Within sex rows, means with different superscript letters differ ($P < 0.05$).
[1]Means are shown as pen averages. CTL = control; RAC = ractopamine-fed.
[2]During phase I, ractopamine HCl was added to the feed at 5 mg/kg and during phase II at 10 mg/kg.

no difference in ADG associated with social rank ($P > 0.10$; data not shown). There was no evidence of social rank effect on slaughter BW ($P > 0.10$).

Live Ultrasound Scanning

Backfat Depth at the 10th Rib. When analyzing individual pig averages for 10th-rib backfat, the interaction of sex × scanning day was significant, with gilts having initially less backfat than barrows ($P < 0.05$; Table 4). When averaging pen scanning measurements for backfat at the 10th rib, a main effect of scanning day was also detected (initial d 0 = 12.0 vs. final d 28 = 15.9 ± 0.30; $P < 0.001$).

On the analyses of mean accretion (final minus initial scanning) in UBFTR, a 3-way interaction of treatment × sex × social rank was statistically significant ($P < 0.05$); this interaction resulted from dominant RAC-fed barrows having increased UBFTR, whereas RAC feeding decreased UBFTR in the other social ranks. Conversely, dominant RAC-fed gilts had decreased UBFTR, whereas gilts from the other social rank groups had no

Table 3. Average daily gain and BW of finishing pigs according to social rank status during a 28-d study

Item	Social rank[1] Dominant	Intermediate	Subordinate	Pooled SEM
Pretreatment				
d −14 BW, kg	77.69	77.97	77.29	0.47
d 0 BW, kg	92.86	93.47	92.28	0.82
Phase I,[2] d 0 to 14				
ADG, kg/d	0.914	0.970	0.895	0.04
d 14 BW, kg	105.66	107.05	104.81	0.973
Phase II,[2] d 14 to 28				
ADG, kg/d	0.916†	1.026†	1.051†	0.04
d 28 BW, kg	118.49	121.42	119.52	1.05
Overall, d 0 to 28				
ADG, kg/d	0.915[a]	0.998[b]	0.973[b]	0.02
Slaughter,[3] d 31				
d 31 BW, kg	119.76	—	121.15	1.19

[a,b]Within treatment rows, means with different superscript letters differ ($P < 0.05$). †Means tend to differ ($P < 0.10$).
[1]Means are shown as individual averages.
[2]During phase I, ractopamine HCl was added to the feed at 5 mg/kg and during phase II at 10 mg/kg.
[3]At slaughter, only the BW of dominant and subordinate pigs were measured.

Table 4. Average live ultrasound scanning measurements of barrows and gilts for initial (d 0) and final (d 28) 10th-rib backfat (UBFTR), last-rib backfat (UBFLR), and loin eye area (ULEA)

	Initial scanning[1]		Final scanning[1]		
Item	Barrow	Gilt	Barrow	Gilt	Pooled SEM
UBFTR, mm	12.79[a]	11.24[b]	15.83	16.03	0.42
UBFLR, mm	14.10[a]	11.19[b]	15.10	16.02	0.50
ULEA, cm^2	33.59	34.34	46.08	46.11	0.59

[a,b]Within rows, means with different superscript letters differ ($P < 0.05$).

[1]Means are shown as pen averages. Overall averages for initial scanning for UBFTR, UBFLR, and ULEA was significantly different than the final scanning ($P < 0.05$).

change in UBFTR due to RAC feeding. Ractopamine-fed pigs belonging to the intermediate social rank had the least UBFTR accretion (1.5 ± 0.5), whereas dominant CTL gilts had the greatest UBFTR accretion (6.4 ± 1.1; $P < 0.05$). No main effects of treatment (CTL = 4.2 vs. RAC = 3.6 ± 0.4) or social rank (dominant = 4.2 ± 0.6; intermediate = 3.1 ± 0.40; subordinate = 4.4 ± 0.6) were detected ($P > 0.10$), but there was a main effect of sex. Barrows had a smaller backfat incremental increase at the 10th rib than gilts (3.0 vs. 4.8 ± 0.4; $P < 0.01$).

Backfat Depth at the Last Rib. The interaction of sex × scanning day was significant, with initial UBFLR being greater in barrows compared with gilts ($P < 0.05$; Table 4), but no sex difference was seen on final ultrasound scanning. The overall average of UBFLR of initial scanning was less than the final scanning (12.0 vs. 15.9 ± 0.1; $P < 0.001$), but no evidence for RAC treatment effect was detected at any of the scanning days ($P > 0.10$). Similarly, at the pen level, main effect of scanning day and interaction of sex × scanning day were significantly different for UBFLR ($P < 0.05$).

As suggested above, backfat depth accretion at the last rib was strongly affected by sex. When comparing results of initial vs. final scanning day, it was found that gilts gained more UBFLR when compared with barrows (4.8 vs. 1.0 ± 0.7; $P < 0.001$). Nonetheless, no treatment effect (CTL = 3.4 vs. RAC = 2.5 ± 0.7; $P > 0.10$), social rank effects (dominant = 2.1 ± 0.9; intermediate = 2.6 ± 0.7; subordinate = 4.1 ± 0.9; $P > 0.10$), or interactions were found significant ($P > 0.10$). When analyzing pen averages for last-rib backfat increments, no main effects or interactions were detected ($P > 0.10$).

Loin Eye Area. The size of loin eye area was smaller at initial compared with final scanning (33.9 vs. 46.1 ± 0.4; $P < 0.001$). However, no main effects of treatment, sex, social rank, or interactions were significant on loin eye size ($P > 0.10$; Table 4). The same patterns were observed when evaluating ULEA as pen averages. Consistently, analyses of changes in loin eye area showed no evidences of treatment (CTL = 11.7 vs. RAC 12.5 ± 0.5), sex (barrow = 12.5 vs. gilt = 11.7 ± 0.5), or social rank effects (dominant = 12.6 ± 0.6; intermediate = 12.2 ± 0.45; subordinate = 11.5 ± 0.6) or their interactions ($P > 0.10$). Means and respective significances for pen averages of ULEA were very similar to individual averages.

Hoof Lesion Scoring

Rear and front hoof lesions such as splits, cracks, and bruises were evaluated at slaughter and presented as counts of average individual lesions (Table 5). There was no evidence of significant interaction of treatment, sex, or social rank effects for hoof lesions ($P < 0.10$). After 4 wk of RAC feeding, pigs had a significantly greater number of total front hoof lesions than did CTL pigs ($P < 0.05$). The types of lesions more frequently observed in the front hooves of RAC pigs were cracks-erosions and bruises ($P < 0.05$). The same lesions were also more evident on rear hooves of RAC-fed compared with CTL pigs ($P < 0.05$), along with total rear hoof lesions ($P < 0.001$). The frequency of observations of rear hoof splits also tended to be greater in RAC pigs ($P = 0.08$).

When analyzing effects of sex on lesion score, it was found that barrows had a greater number of front hoof lesions, including cracks-erosions ($P < 0.01$) and potentially more bruises ($P = 0.08$). Barrows also tended to have more rear hoof bruises than gilts ($P = 0.07$). Moreover, dominant pigs had a greater number of front cracks and rear splits on the hooves than subordinates ($P < 0.05$). Frequency of total lesions in dominant pigs was greater in rear hoof ($P < 0.05$) and tended to be greater in the front hooves ($P = 0.09$), when compared with subordinate pigs. The average of the sum of total rear and front hoof lesions of animals fed RAC (treatment effect, $P < 0.001$), barrows (sex effect, $P < 0.01$), and dominant pigs (social rank effect, $P < 0.05$) were observed in greater occurrences at slaughter (Table 5).

Bacteria Monitoring

All samples were analyzed for S*almonella* presence, but no colonies were detected at any of the sampling days during the experiment. The interaction of treatment × sampling day was found as a trend toward significance (treatment × sampling, $P = 0.08$), and details are shown in Table 6. The RAC-fed pigs had

Table 5. Front and rear hoof lesions scored in finishing pigs at the time of slaughter, shown in relation to the effects of ractopamine feeding, sex, and social rank status

	Treatment[1]		Sex[1]		Social rank[1]		
Lesion types	CTL	RAC	Barrows	Gilts	Dominant	Subordinate	Pooled SEM
Front splits	1.37	1.56	1.69	1.25	1.56	1.37	0.23
Front bruises	0.75[a]	1.50[b]	1.44†	0.81†	1.25	1.00	0.25
Front cracks-erosions	1.44[a]	2.19[b]	2.31[c]	1.31[d]	2.19[e]	1.44[f]	0.24
Total front lesions	3.56[a]	5.25[b]	5.44[c]	3.37[d]	5.00†	3.81†	0.49
Rear splits	1.25†	1.87†	1.56	1.56	1.94[e]	1.19[f]	0.24
Rear bruises	1.25[a]	2.25[b]	2.12†	1.37†	2.00	1.50	0.28
Rear cracks-erosions	1.62[a]	2.50[b]	2.06	2.06	2.00	2.12	0.27
Total rear lesions	4.12[a]	6.62[b]	5.75	5.00	5.94[e]	4.81[f]	0.37
Average of total lesions[2]	3.84[a]	5.94[b]	5.59[c]	4.19[d]	5.47[e]	4.31[f]	0.30

[a,b]Within treatment row, means with different superscript letters differ ($P < 0.05$). †Means tend to differ ($P < 0.10$).

[c,d]Within sex row, means with different superscript letters differ ($P < 0.05$). †Means tend to differ ($P < 0.10$).

[e,f]Within social rank row, means with different superscript letters differ ($P < 0.05$). †Means tend to differ ($P < 0.10$).

[1]Means are shown as individual averages. CTL = control; RAC = ractopamine-fed.

[2]Determined by averaging total front and rear hoof lesions.

Enterobacteriaceae concentrations declining over time, as observed that shedding at d 18 and 25 was less than at d 4 ($P < 0.05$). Meanwhile, CTL pigs had a much slower reduction in Enterobacteriaceae and were nearly stable throughout the 28-d study (Table 6). The overall average for Enterobacteriaceae shedding was greater in RAC compared with CTL finishing pigs ($P < 0.05$; Table 6). There was also a main effect of day of sampling on fecal Enterobacteriaceae ($P < 0.001$), with d 4 showing the greatest concentrations followed by d 11. A main effect of sex was additionally found, with gilts shedding greater concentrations of Enterobacteriaceae compared with barrows (6.42 vs. 6.12 ± 0.10; $P < 0.05$), and no interactions with sex were observed. There was no evidence of an effect of social rank on fecal Enterobacteriaceae concentrations (dominant = 6.36 ± 0.14; intermediate = 6.22 ± 0.10; subordinate = 6.23 ± 0.14), nor interactions involving social rank ($P > 0.10$).

Bacteria Monitoring at Slaughter. There was no evidence of *Salmonella* presence in any of the intestinal contents collected from ileum, cecum, and rectum at slaughter of the dominant and subordinate pigs from each pen. The overall average of rectal Enterobacteriaceae shedding in samples collected from finishing pigs at slaughter did not differ from those of d 25 (5.62 ± 0.20 vs. 5.79 ± 0.15, respectively; $P > 0.10$), which was the last fecal sampling before slaughter. However, at slaughter, a treatment effect was found for this variable. Ractopamine-fed pigs had decreased concentrations of cecal and rectal Enterobacteriaceae than CTL pigs at the slaughter day ($P \leq 0.05$; see Table 7). Sex × social rank only tended to be significant for cecal Enterobacteriaceae concentration ($P = 0.07$), with dominant barrows having the greatest cecal Enterobacteriaceae (5.88 ± 0.21), followed by subordinate gilts (5.74 ± 0.19), dominant gilts (5.71 ± 0.21), and subordinate barrows (5.15 ± 0.21). Sex did not affect cecal Enterobacteriaceae counts ($P > 0.10$). Regarding the ileal Enterobacteriaceae counts, no main effect of treatment was detected ($P > 0.10$), although a treatment × social rank interaction was observed ($P < 0.05$; Table 7). Furthermore, subordinate RAC-fed pigs had the greatest ileal Enterobacteriaceae concentrations (6.45 ± 0.35), whereas CTL subordinate pigs had the least ileal concentrations (5.50 ± 0.44); dominant RAC-fed and dominant CTL pigs had, respectively, 5.58 and 6.41 ± 0.35 Enterobacteriaceae concentrations. However, due

Table 6. Enterobacteriaceae fecal shedding concentrations collected from control and ractopamine-fed finishing pigs once a week for 5 consecutive weeks

Item	Sampling day	CTL[1]	RAC[1]	Pooled SEM
Pretrial	−3	5.90[bc]	6.23[bc]	0.23
Phase I,[2] d 0 to 14	4	6.43[abc]	7.31[a]	0.22
	11	6.19[bc]	6.66[ab]	0.21
Phase II,[2] d 14 to 28	18	6.15[bc]	6.20[bc]	0.20
	25	5.92[bc]	5.67[c]	0.20
Overall, 28 d		6.12[d]	6.41[e]	0.10

[a–c]Within a column, means with different superscript letters differ ($P < 0.05$).

[d,e]Within a row, means with different superscript letters differ ($P < 0.05$).

[1]Means are shown as individual averages as $\log_{10}$ cfu/g of feces. CTL = control; RAC = ractopamine-fed.

[2]During phase I, ractopamine HCl was added to the feed at 5 mg/kg and during phase II at 10 mg/kg.

Table 7. Concentrations of Enterobacteriaceae measured from intestinal content of control and ractopamine-fed finishing pigs at the time of slaughter

Sampling location	CTL[1]	RAC[1,2]	Pooled SEM
Rectum	6.00[a]	5.25[b]	0.23
Cecum	5.83[a]	5.41[b]	0.14
Ileum	5.95	6.02	0.28

[a,b]Within a row, means with unlike subscript letters differ ($P < 0.05$).

[1]Means are shown as individual averages as $\log_{10}$ cfu/g of feces. CTL = control; RAC = ractopamine-fed.

[2]During phase I, ractopamine HCl was added to the feed at 5 mg/kg and during phase II at 10 mg/kg.

to lack of contents, 10 (CTL = 6 and RAC = 4) out of the 32 ileal samples were not collected at slaughter and were therefore missed for the statistical analysis.

DISCUSSION

The current study examined effects of RAC feeding, delivered as a “step-up” program, and also considered the sex and social rank status of animals. Although extensive research has reported the RAC-enhancing effects on growth performance variables, a very limited number of studies examined the consequences of RAC feeding on gut microbial ecology and none have explored its effect on hoof soundness or the potential interaction of social rank with these investigative areas. Initial RAC feeding did not affect any of the growth variables investigated. Positive effects of RAC feeding on growth were more evident during phase II after raising the RAC feeding dose to 10 mg/kg. It is known that RAC begins to enhance growth performance variables between 7 and 14 d of feeding (Schinckel et al., 2002). Thus, the increased degree of RAC feeding may compensate for the reduction of the effective response of the compound over time that is caused by either desensitization or downregulation of β_1-adrenergic receptors for which RAC has a greater affinity (Moody et al., 2000; Mills and Spurlock, 2003). These findings are consistent with other studies reporting greater ADG, G:F, and better profitability when applying the same 5 to 10 mg/kg of RAC “step-up” feeding program (Schinckel et al., 2002; See et al., 2004). Consistent with previous reports, an overall increase in BW, ADG, and G:F, with no changes in ADFI, was observed in RAC-fed pigs (Gu et al., 1991a,b; Schinckel et al., 2002). Furthermore, barrows were constantly heavier then gilts and had greater ADFI, whereas gilts had greater overall G:F throughout the study, agreeing with previous findings (See et al., 2004; Groesbeck et al., 2007). It was also our interest to evaluate effects of social rank status of pigs on some growth performance variables, because it has been suggested that subordinate pigs visit the feeder less frequently by being physically displaced by greater rank status individuals (Young and Lawrence, 1994), which can lead to less BW gain. However, in our study, dominant pigs, especially dominant barrows, showed less ADG than the intermediate and subordinate pigs, indicating lower social ranking pigs may compensate intake and thus BW gain by feeding at alternative times such as during the night.

The initial UBFTR and UBFLR were less in gilts than barrows, but this difference disappeared by the final scanning, 28 d later. These results partially disagree with previous studies reporting that gilts have less final 10th-rib backfat than barrows (See et al., 2004; Groesbeck et al., 2007). Nevertheless, the greater UBFTR and UBFLR accretions detected in gilts may be linked to the increase in ADFI that gilts had showed between phases I and II, compared with decreasing ADFI of barrows. Other studies have shown that RAC decreases UBFTR in finishing pigs (See et al., 2004; Carr et al., 2005), but this effect was not clearly observed in the present study and was also not seen by Groesbeck et al. (2007). There was only an indication of social rank effect on UBFTR, because dominant RAC-fed barrows had the greatest UBFTR. There was no statistical evidence of social rank affecting UBFLR, but still, subordinate individuals had greater UBFLR than dominant and intermediate pigs. Furthermore, according to our findings, RAC feeding did not affect ULEA, opposing previous studies reporting that RAC increased ULEA in pigs on various feeding programs, including the one applied in the current study (See et al., 2004; Carr et al., 2005; Groesbeck et al., 2007).

Similar negative effects on hoof soundness caused by the β-adrenergic salbutamol (Penny et al., 1994) were also observed in finishing pigs fed RAC in this study. As scored at slaughter, RAC-fed animals had notably more front and rear hoof lesions including sand cracks, erosions, and bruises and nearly twice as many overall hoof lesions compared with control individuals. When salbutamol was fed at 1 to 5 mg/kg for up to 28 d, there was an increase in the frequency and severity of foot lesions in finishing pigs, with severity of lesions being proportional to the dose (Penny et al., 1994). Following the same mechanism of salbutamol (Penny et al., 1994), as a compound from the same category, RAC may also be interfering with horn production causing animals to be more vulnerable to hoof damage. During our experiment, only 3 pigs were therapeutically treated for lameness, with only one of them originating from the RAC-fed group (data not shown). Hoof lesions at slaughter were also observed with greater frequency in front hooves of barrows. Supplementation with biotin, which was also provided in the diet of the experimental animals, has been shown to decrease the incidence of heel cracks and erosions in pigs (Bryant et al., 1985). Biotin and other vitamins and minerals do not compensate for the negative effects of heavy BW on hoof soundness; in our experiment, barrows were heavier than gilts throughout the entire experiment. This factor can be aggravated when associated with flooring structure. Concrete slats, particularly slats with chipped and sharp edges, and rough flooring can increase predisposition of finishing pigs to more severe hoof lesions (Newton et al., 1980; Mouttotou et al., 1999). As observed in the present study, dominant pigs presented more rear hoof problems than subordinate pigs, but live BW was similar between social ranks. The connection of social rank status and incidence of hoof lesion is intriguing but could easily be associated with fighting within the pen, because dominant individuals may engage more frequently in agonistic interactions to maintain their social status. It may also be postulated that subordinate pigs move less to avoid undesired encounters with dominant individuals, thus being less predisposed to hoof damage. Several nutritional factors have been linked to hoof unsoundness in pigs, and normal balanced diets, that meet NRC (1998) specifications, allow adequate growth but may not prevent such problem. The diet provided to the experimental animals was in accordance with NRC (1998) requirements.

A previous study by Marchant-Forde et al. (2003) showed that there are consequences of using RAC as a feed additive on the behavior and physiology of pigs subjected to handling and transportation. Some of the physiological alterations reported include elevated concentrations of epinephrine and norepinephrine, which have been linked to increased in vitro growth and virulence of *Escherichia coli* and *Salmonella enterica* (Lyte et al., 1997; Bailey et al., 1999; Kinney et al., 2000; Green et al., 2004). Our hypothesis was that animals fed a diet containing RAC, and consequently with greater concentrations of catecholamines, would carry greater concentrations of bacteria in their intestinal tract, particularly *Salmonella* and *E. coli.* To the best of our knowledge, this is the first study on the potential effects of RAC on Enterobacteriaceae (which is predominantly composed by generic *E. coli*) in swine. Nevertheless, we were not able to detect *Salmonella* in any of the fecal and slaughter samples collected during this study, which were conducted under natural conditions (i.e., no artificial infections or inoculations). Edrington et al. (2006b) recently reported a decrease in the frequency of *Salmonella* shedding in experimentally inoculated pigs subjected to a much greater in-feed RAC dose (20 mg/kg) and for an extended period of time (38 d). The same authors (Edrington et al., 2006a), however, reported a tendency of RAC to increase *Salmonella* and decrease *E. coli* (specifically the serotype O157:H7) shedding in beef cattle. The conflicting reports, based on limited studies conducted with different species, in addition to the potential food safety implications of a feed additive used immediately before slaughter, constitute solid arguments in favor of further research. As observed in this study, overall, RAC-fed pigs consistently shed greater Enterobacteriaceae concentrations than CTL pigs, with peak concentrations found at the end of the first week (d 4) of a RAC-administered ration. From the peak concentration detected at d 4 until slaughter, RAC-fed pigs decreased Enterobacteriaceae shedding more markedly than did CTL individuals (RAC decreased 1.65 log cfu/g of feces, whereas CTL pigs decreased only 0.51 log cfu/g of feces).

At slaughter, RAC pigs had even less concentrations of Enterobacteriaceae in rectal and cecal luminal contents. The patterns of Enterobacteriaceae shedding concentrations and gut populations found in this study, although interesting from a food safety standpoint, are difficult to explain not only due to their complexity but also due to the lack of information and knowledge about the potential effects of RAC on the intestinal microbial ecology. However, the fact that RAC-fed pigs had significantly fewer Enterobacteriaceae concentrations at slaughter suggests that this feed additive may have an effect on the intestinal bacteria load of finishing pigs entering the abattoir. Moreover, the greater variability in Enterobacteriaceae shedding found within the treatment group suggests a link between intestinal microbial populations and RAC-associated increasing susceptibility of pigs to stressful events, such as handling and environmental changes (Marchant-Forde et al., 2003). Thus, the potential for RAC influencing intestinal microbial populations and fecal shedding of Enterobacteriaceae warrants further research.

Interestingly, gilts and barrows differed regarding Enterobacteriaceae shedding, with gilts consistently shedding greater concentrations throughout the experiment. The effect of sex on different types of stress responses and consequences have been demonstrated in other species, with females being more vulnerable than males (Drossopoulou et al., 2004; Dalla et al., 2005; Kiank et al., 2006). Therefore, we postulate that gilts may be more susceptible to environmental changes and stress, which in turn may alter intestinal microbial populations. However, it has to be kept in mind that there may also be a combined effect of social rank. At slaughter, cecal Enterobacteriaceae populations were found to be greatest in dominant barrows and least in subordinate barrows, with dominant and subordinate gilts carrying intermediate concentrations. In stable social hierarchies, subordinate pigs are more susceptible to diseases, which lead to greater mortality or morbidity, or both, rates upon challenging events of pathogen (Hessing et al., 1994). In the current study, RAC-fed subordinate pigs and CTL dominant animals had greater concentrations of Enterobacteriaceae in the ileal contents collected at slaughter. Although the 10 ileal luminal content samples reported missing from the study were balanced for treatment, sex, and social rank, ileal results should still be interpreted with caution, because the total sampling was smaller than the number of samples collected from the other intestinal portions.

In summary, RAC-fed pigs, especially barrows, showed enhanced growth performance with a weak interference of social rank on growth. Despite the positive effects of RAC feeding on production performance, pigs fed the compound had more front and rear hoof lesions; likewise, barrows and dominant individuals had a greater occurrence of hoof lesions. *Salmonella* shedding was not detected, and pigs fed RAC peaked Enterobacteriaceae shedding concentrations at the first week of RAC feeding but progressively decreased up to slaughter. Dominant social rank may be associated with greater concentrations of cecal Enterobacteriaceae in barrows, and RAC feeding may lead to a decrease in ileal concentrations of Enterobacteriaceae in dominant animals while increasing in subordinate pigs. Nevertheless, additional research is needed to explore the link between RAC feeding on hoof soundness and Enterobacteriaceae populations in the gastrointestinal tract of finishing pigs. Sex and social rank should also be taken into consideration for future studies, because they may affect treatment responses.

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